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EXPERIMENTAL STUDY OF SURVIVAL RATE OF A DIPHTHERIC BACILLUS IN AEROSOL

by

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EXPERIMENTAL STUDY OF SURVIVAL RATE OF A DIPHTHERIC BACILLUS IN AEROSOL

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The problem of seeking new means to combat airborne droplet infections is of interest to specialists of various areas; it is of equal interest to hygienists, microbiologists, contagious disease specialists, epidemiologists, and others. As a result of this, the study of bacterial aerosols is of common interest because microorganisms dispersed in the air are a model of the basic element of the airborne mechanism in infection transmission. Using this model, each scientific area solves the problems posed by this model.

However, no matter what problems are encountered in the study of aerosols, microorganism survival rate is almost always the basic test which describes the state of airborne microflora. Unfortunately, thus far, very few individuals have been able to study the survival rate in an aerosol per se, due to the fact that in the majority of cases the obtained results were the combined product of the biological dying-off process and a purely physiological disappearance of particles as a result of aerosol settling. In studying the bacterial aerosols, the works of R. M. Ferri with coauthors, et al., carried out on monodisperse systems, are included in a number of most successful attempts to resolve this difficulty.

However, in a natural environment the bacterial aerosols, as a rule, are polydisperse systems. In polydisperse systems the particles are of varied dimensions; accordingly, larger particles contain more microbes than the smaller. This peculiarity of structure of polydisperse systems was noted and studied by L. Sonkin who introduced a proportional mathematical relationship between the particle size, concentration of the dispersed bacterial suspension and number of microbes in a particle.

In a polydisperse system the larger particles and therefore particles more saturated with microbes settle faster than the small particles containing less microbes. As a result of this the average size of aerosol particles decreases gradually. The total concentration of particles also decreases (the so-called rated aerosol concentration). Thus, in order to "free" the survival rate index of change of the airborne microflora from misrepresentation as a result of settling, it is necessary to introduce at least two corrections: one of them should be associated with changes in the rated aerosol concentrations, the other - with changes in the average particle size. It is convenient to replace the latter with an equivalent quantity of the average number of microbes in the particle.

We carried out experiments dealing with the survival rate in a chamber designed by us, which is known under the name KIEM-5 apparatus. The live microbe concentration in the air was determined by means of granular filters of the finest sodium alginate powder. The rated aerosol concentration was determined in the VDK instrument (B. V. Deryagin and G. Ya. Vlasenko). The average number of microbes in the particle was determined by counting them on the preparations on which the aerosol settled, under the microscope. The settling out was done in an electrostatic precipitator of our own design, fed by high-voltage generator of the R. A. Boytsekhovskiy system.

Thus, the survival rate in a polydisperse aerosol was established on the basis of a simultaneous study of aerosols, using three methods: selection of specimen for bacterial seeding, determination of rated concentration, and calculation of the average number of microbes in the particle.

However, this method can be employed if fractions of particles, free of microbes, are absent in the experimental aerosol model. As shown by the theoretical studies carried out by us, such fractions can form during the spraying of bacterial suspensions. In this case they comprise the most finely dispersed portion of the aerosystem and can be missed during the microscopy of particle deposits. At the same time, being noticeable in the VDK instrument, they can distort the actual survival rate determination result.

Using the theoretical method it was also determined that the microbe-free fraction, when using the usual sprayers, cannot form in the suspension whose concentration is greater than $1.92 \cdot 10^9$ cells per 1 ml.

Another limitation is the case when more than 40-50 cells accumulate in the largest particles, when it becomes impossible to count them.

Based on this premise we used the experimental aerosol models of the diphtheric bacillus obtained by spraying suspensions having the concentration of 10-14 billion microbes per 1 ml. We characterized the diphtheric rod survival rate in the form of a series of percent ratios taken relative to the first determination of degree of survival rate in the aerosol after its formation.

In order to study the survival rate of the diphtheric bacillus (strain PW-8) directly in the air, the microbes were washed three times in distilled water and sprayed as an aqueous suspension. The rapid evaporation of water results in the fact that the cells are directly suspended in the air. At room temperature and average humidity (61%) the survival rate of the diphtheric bacillus over a period of 2 h decreases to 20% of the original value on the average. The rate of this process is $5.7 \cdot 10^{-3}$ (Fig. 1). In varying the humidity over the range from 40 to 90% (keeping the temperature constant), we were unable to observe any significant deviations in the survival rate from that which was established at 61%. On the other hand, varying the temperature but keeping the humidity constant (61%), there is a considerable change in the survival rate. Thus, at temperature of -6° [sic] the survival rate decreased to only 80% in 2 h and the dying-off rate comprised a relatively small value ($0.5 \cdot 10^{-3}$). However, at a temperature which approaches the optimum for growth, i.e., at 35°, the dying-off rate of microbes raises sharply and the rate c this process reaches $16.9 \cdot 10^{-3}$-$34.6 \cdot 10^{-3}$ (Fig. 2).

In spraying the diptheria based on saliva, i.e., in the case when between the cells and the air there is another substance - mucouse and protein substances of saliva - the survival rate curve changes its form considerably (Fig. 3). For the first 45 min the

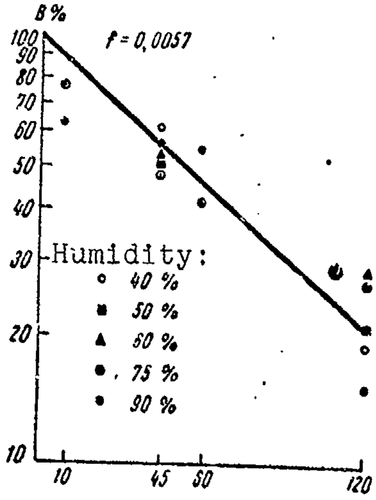

Fig. 1. The diphtheric bacillus dying off curve, sprayed on water at 18°.

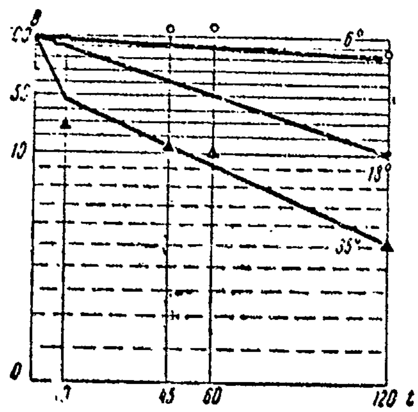

Fig. 2. Dying off of the diphtheria microbe at 6, 18 and 35° with 61% air humidity.

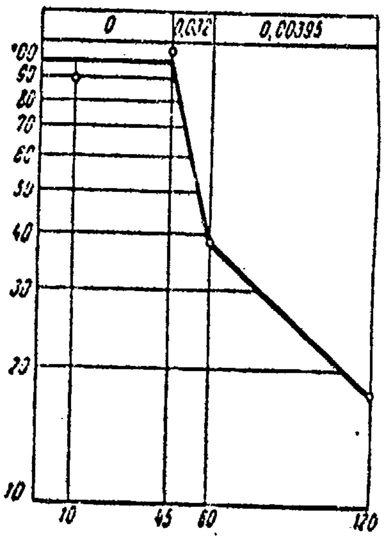

Fig. 3. Survival rate of a diphtheric bacillus in a polydisperse aerosol based on saliva (18° and 61% humidity).

decrease in the survival rate is negligible, the dying-off rate is almost zero. Then the dying-off process rises sharply, survival rate drops and at the end of 2-h exposure the dying-off rate approaches that observed in aerosols obtained by spraying of the aqueous suspension. Thus, when spraying the suspension with saliva $K = 3.9 \cdot 10^{-3}$ and with aerosol based on aqueous suspension $K = 5.7 \cdot 10^{-3}$. The coincidence is even more accurate between the dying-off rate in the second hour of exposure and that in the systems based on aqueous suspensions in the aerosols made of broth suspensions.

Figure 4 shows the aerosol after a 2-h exposure to air. The majority of particles in Fig. 4 has clear signs of drying out, manifested in the thickening of cellular composition of the particle, disappearance of space between cells filled with saliva. This forces us to assume that the increase in dying off in the aerosol with saliva, observed an hour after its appearance, is associated with the drying out process proceeding directly to the cells. Evidently, such materials as saliva or broth, which lower the evaporation rate of water, protect the cells from evaporation for a certain period (depending on the particle size of the aerosol). When the water evaporates from the broth or saliva, the moisture begins to evaporate from the bacteria cells themselves, thereby starting their rapid dying-off process.

CONCLUSIONS

1. A formula was developed for calculating the survival rate in polydisperse aerosols, which excludes the effect of particle sedimentation on the final result; the limits of its application are determined.

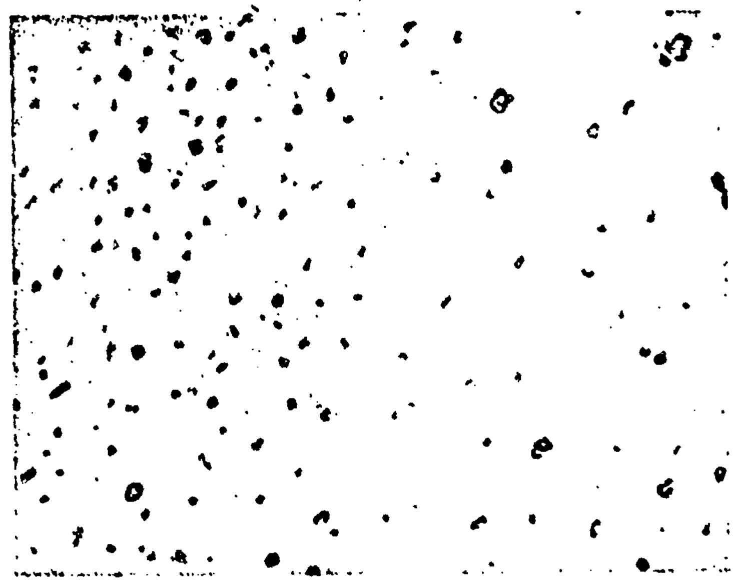

Fig. 4. Aerosol particles after a two-hour exposure to air.

2. Significant sensitivity of diphtheric bacillus to the changes in temperature is clarified; at a temperature below zero the causative agent dies very slowly, while at 35° its survival rate is sharply reduced; at 18° the survival rate of a dephtheric bacillus is in the intermediate position.

3. Saliva and broth protect the microorganisms from drying up for a certain period by slowing down the evaporation. This ensures a high survival rate of the diphtheria causative agent for the first 45 min of aerosol existence.